ERJ Express. Published on September 17, 2008 as doi: 10.1183/09031936.00094808

Long-term effects of bosentan in patients with HIV-associated pulmonary arterial hypertension

Short title: Bosentan in PAH associated with HIV

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Word count (excluding abstract, references, tables and legends): 3,341

Funding: This study was supported in part by an educational grant from Actelion Pharmaceuticals France.

Abstract

Bosentan has proven 4-month efficacy in patients with human immunodeficiency virus-associated pulmonary arterial hypertension (PAH-HIV). Here, we describe the long-term outcome of unselected PAH-HIV patients treated with first-line bosentan.

Data were analysed for 59 consecutive WHO functional class II-IV PAH-HIV patients treated with first-line bosentan between May 2002 and July 2007. HIV status, 6-minute walk distance and haemodynamics were assessed at baseline, after 4 months, and every 6-12 months thereafter.

After 4 months, 6-minute walk distance increased from 358±98 to 435±89m and pulmonary vascular resistance decreased from 737±328 to 476±302 dyn·s·cm^{-5}. At last evaluation, 29±15 months, 6-minute walk distance remained stable and pulmonary vascular resistance decreased further to 444±356 dyn·s·cm^{-5}. Haemodynamics normalised in 10 patients. At their last evaluation, these 10 patients were in WHO functional class I, with a 6-minute walk distance of 532±52m. Overall survival estimates were 93%, 86% and 66% at 1, 2 and 3 years, respectively. Bosentan was safe when combined with highly-active antiretroviral therapy with no negative impact on HIV-infection control.

Our data confirm long-term benefits of bosentan therapy in PAH-HIV patients with improvements in symptoms, 6-minute walk distance and haemodynamics, and with favourable overall survival.

Keywords: Bosentan – endothelin – human immunodeficiency virus – hypertension, pulmonary

Introduction

Pulmonary arterial hypertension (PAH) is a life-threatening complication of human immunodeficiency virus (HIV) infection.[1] The prevalence of PAH in HIV-infected individuals, which is estimated to be 0.5%, is at least 1000-fold higher than the prevalence of idiopathic PAH (IPAH) in the general population and does not appear to have changed over recent decades.[2] HIV-infected patients represented 8.1% of PAH patients in the 2003-2004 French Registry.[3] The mechanisms underlying the link between HIV infection and PAH have not yet been established. HIV infection increases the risk of development of PAH regardless of the routes of infection, the stage of HIV infection and the degree of immunodeficiency.[4,5] Evidence suggests that the presence of PAH has a significant influence on mortality and, in most cases, death is causally related to PAH rather than to other complications of HIV infection.[5,6]

The latest treatment guidelines stress that adequate evidence to support the optimal management of PAH associated with HIV (PAH-HIV) is lacking.[7] There are still conflicting data on the impact of highly-active antiretroviral therapy (HAART), with some reports suggesting that there is no effect of HAART on the presence or severity of PAH,[8] and others reporting that HAART reduces mortality associated with PAH-HIV.[9] In the absence of specific recommendations, PAH-HIV treatment follows guidelines for IPAH treatment.[7] Acute pulmonary vasodilator response is rare in patients with PAH-HIV and therefore long-term calcium channel blocker treatment is likely to be inadequate in such patients.[3,10] Three classes of drugs are currently approved as "specific therapy" to treat patients with PAH: (i) prostacyclin analogues administered via intravenous (epoprostenol) or subcutaneous (treprostinil) routes, or by inhalation (iloprost); (ii) the oral dual endothelin receptor antagonist bosentan and (iii) the phosphodiesterase type 5 inhibitor (PDE5i)

sildenafil. The results from small studies suggest that treatment with continuous intravenous infusion of epoprostenol[5,11] and inhaled iloprost[12] may provide benefits for patients with PAH-HIV. However, there are concerns about the long-term use of these drugs,[5] especially epoprostenol, which needs a permanent central venous access with the associated potential for infectious complications, particularly in immunocompromised patients. Some studies have reported the efficacy of sildenafil in patients with PAH-HIV[13,14]; however, drug-drug interactions with protease inhibitors may limit the use of sildenafil in these patients.[15] The selective oral endothelin receptor antagonist, ambrisentan, appears to have short-term benefits in patients with PAH;[16] however, data from patients with PAH-HIV are as yet either lacking or unpublished. Finally, an open "proof of concept" 4-month study involving 16 patients with PAH-HIV suggested that bosentan improves exercise capacity, quality of life and haemodynamics, without a negative impact on the control of HIV infection.[17]

Because of the short-term beneficial results observed with bosentan in patients with PAH-HIV,[17] we aimed to investigate the potential benefit and safety of long-term treatment in a larger group of patients treated with first-line bosentan.

Materials and methods

Study subjects

We evaluated 59 consecutive patients with PAH-HIV treated with first-line bosentan between May 2002 and July 2007 at the Antoine Béclère Hospital (Clamart, France) and Rangueil-Larrey Hospital (Toulouse, France). Of these patients, 12 were previously described in a 16-week pilot study conducted in 16 patients to evaluate first-line therapy with bosentan in PAH-HIV;[17] 4 of these 16 patients were from centres other than Antoine Béclère Hospital and were therefore not included in the present report. All patients had HIV infection and symptomatic PAH in World Health Organization (WHO) functional class (FC) II-IV. PAH was diagnosed by means of right heart catheterisation (RHC) showing a mean pulmonary arterial pressure (mPAP) at rest >25 mmHg, a pulmonary capillary wedge pressure (PCWP) <15 mmHg and a pulmonary vascular resistance (PVR) >250 dyn·s·cm^{-5}. Acute vasodilator testing with inhaled nitric oxide (10 ppm) was performed in all patients, as previously described.[18] Primary lung disease and post-embolic PAH were ruled out by pulmonary function tests, computed tomography and ventilation/perfusion ratios.

According to French legislation, ethics committee agreement and provision of informed consent are not required for retrospective collection of data corresponding to current practice. The database was, however, complied anonymously with the restrictive requirements of the 'Commission Nationale Informatique et Liberté', the organisation dedicated to privacy, information technology and civil rights in France. This study was approved by the local Institutional Review Board.

Study design

The goal of this retrospective study was to describe the long-term outcome of unselected patients with PAH-HIV treated with first-line bosentan.

Methods

All patients received non-specific supportive therapies in accordance with current guidelines, including oral anticoagulants to maintain an international normalised ratio of 1.5-2.5 unless contraindicated, diuretics to control signs and symptoms of right heart failure including peripheral oedema, and long-term oxygen therapy if hypoxaemia was present.[19] Patients received a regimen of HAART if required according to the most recent recommendations.[20] Bosentan was prescribed at 62.5 mg twice daily for 4 weeks followed by 125 mg twice daily thereafter. Liver function tests were performed every 2 weeks during the first 6 weeks and monthly thereafter. In the case of elevated liver enzymes bosentan was stopped or the dosage reduced in accordance with current recommendations.[21]

All patients had a complete baseline evaluation before starting bosentan therapy, including assessment of modified WHO FC, physical examination, routine blood tests and non-encouraged 6-minute walk test (6MWT) according to the American Thoracic Society recommendations.[22] WHO FC assessment, non-encouraged 6MWT, and RHC were reassessed after 4 and 12 months of bosentan therapy. Non-invasive assessments were repeated every 4-6 months thereafter and RHC once a year.

At our centres, an additional specific therapy was indicated when the patient was in WHO FC IV on treatment or persisted in WHO FC III after at least 4 months of treatment together with (1) a 6-minute walk distance (6MWD) <250m, (2) a decrease in 6MWD >10% from the previous value in two tests performed at least 2

weeks apart, or (3) a cardiac index (CI) <2.2 $L \cdot min^{-1} \cdot m^{-2}$.[23] Some patients in WHO FC IV received bosentan monotherapy because they refused intravenous epoprostenol.

Analysis

Data were analysed using Statview, version 5.0 (SAS Institute, Cary, NC). Results were expressed as mean ± standard deviation (SD) or as median with the range. Baseline and post-baseline values for 6MWD, haemodynamic parameters and HIV status were compared using a two-sided paired Student's t-test. Only patients having received bosentan monotherapy for one year or more were considered for the last evaluation analysis. For the subgroup of patients in which baseline data, data after 4 months and data at last evaluation were available, the comparisons were made using repeated-measures analysis of variance (ANOVA). Changes in WHO FC were compared using the Chi-squared test. A P-value of <0.05 was considered statistically significant.

Analyses of overall survival and event-free status were performed using an intention-to-treat approach. The date of initiation of bosentan therapy was the starting point to determine the follow-up duration and to estimate survival. Event-free status was defined as survival without lung transplantation, need of prostacyclin analogues and/or PDE5i therapy, discontinuation of bosentan, or acute right heart failure requiring hospitalisation for intravenous diuretics and/or dobutamine infusion. Patients lost to follow-up were censored as of the date of the last bosentan prescription. The Kaplan-Meier method was used to estimate overall survival and event-free status at each time point. For patients with more than one event, only the first event was used in the Kaplan-Meier analysis.

Univariate analysis based on the proportional hazards model was used to examine the effect on survival of selected demographic parameters, medical history, portal hypertension, HIV status and haemodynamic variables assessed at baseline. For continuous variables, we chose to separate patients into two groups on both sides of the median value. Results were expressed as hazard ratios with 95% confidence intervals.

Results

Baseline patient characteristics

Baseline demographics, HIV status and haemodynamics of the 59 patients are described in Table 1. Nineteen patients (32%) had HIV acquired from intravenous drug use, 20 (34%) via heterosexual contact, 16 (27%) via homosexual contact, and 5 (7%) via other causes. At the time of PAH diagnosis, the majority of patients (83%) were receiving HAART, comprising at least three antiretroviral medications, including protease inhibitors and/or non-nucleoside reverse transcriptase inhibitors or both. Thirty patients had co-infection with hepatitis C (n=24) or B (n=6) virus. Of these patients, 10 had mild cirrhosis (Child-Pugh A class). Baseline clinical characteristics and haemodynamics did not differ between patients with and without chronic viral hepatitis, or between patients with and without cirrhosis.

Exercise capacity and haemodynamics after 4-months of bosentan

Fifty-six out of 59 patients were evaluated after 4-months of bosentan monotherapy (Table 2). The remaining three patients either died (n=2), or had bosentan stopped because of an increase of liver enzymes (n=1). Among these 56 patients, WHO FC improved in 45 and remained stable in 11. The 6MWD increased by a mean of 74±88m (Table 2); improving in 47 out of 56 patients. Pulmonary haemodynamics significantly improved, with a 19% increase in CI, an 18% decrease in mPAP, and a 35% decrease in PVR.

Exercise capacity and haemodynamics at last evaluation

Thirty eight out of the 56 patients who were evaluated at 4 months were re-evaluated after 29±15 months (range 12-67 months) on bosentan monotherapy

(Table 2). The remaining 18 patients did not undergo long-term evaluation for the following reasons: 11 were receiving bosentan monotherapy but had not been treated for at least one year at cut-off, four died between 4 and 12 months (all were taking bosentan monotherapy) and one received sildenafil in addition to bosentan. Bosentan was stopped in two additional patients because of an increase of liver enzymes. Of these remaining 38 patients on bosentan monotherapy for at least one year, WHO FC improved in 29 and remained stable in 9 patients at last evaluation compared to baseline. Compared to the 4-month evaluation, 6MWD and CI remained stable, and there was a further decrease in mPAP and PVR (Table 2).

Notably, haemodynamics were normalised in 10 of the 59 patients who received first-line bosentan monotherapy. Individual data for these 10 patients are shown in Table 3. At the time of last evaluation (32±22 months), all 10 patients were in WHO FC I, with a significant improvement in the 6MWD and normalisation of haemodynamic parameters. At the start of bosentan therapy, 3 of these patients were in WHO FC II, five were in FC III and two were in FC IV. Baseline haemodynamic parameters, viral load and $CD4^+$ lymphocyte count of these 10 patients were not different from the baseline values of the 28 remaining patients for whom haemodynamics were not normalised on long-term bosentan monotherapy. In the subgroup of 10 patients for whom hemodynamics were normalised, viral load did not significantly decrease at last evaluation compared with baseline values, while $CD4^+$ lymphocyte count significantly increased in the meantime (Table 3). Conversely, in the subgroup of 28 patients for whom haemodynamics were not normalised, viral load significantly decreased from 2.0±2.5 to 1.3±1.6 log_{10}/mm^3 ($p=0.02$) and $CD4^+$ lymphocyte count increased from 409±358 to 467±360 cells/mm^3 however the difference was not significant ($p=0.32$). Before starting bosentan, 8 patients received HAART and 9 had a $CD4^+$ lymphocyte count

>200/mm^3. At the time of last evaluation, all were on HAART with a CD4$^+$ lymphocyte count >200/mm^3. The number of patients with undetectable viral load remained stable during the follow-up (8/10 at baseline *vs* 7/10 at last evaluation). In one patient, bosentan was permanently interrupted due to reversal of PAH after 2 years of treatment. A 6-month follow-up of this patient showed that normalised haemodynamics and WHO FC I were preserved off-treatment.

Evolution of HIV status

At baseline, 49 out of 59 patients (83%) were receiving HAART at the time of PAH diagnosis. At this time, 51% had undetectable viral load and 80% had a CD4$^+$ count >200/mm^3 (Table 1). After 4 months of bosentan monotherapy, 47 out of 56 patients (84%) were still receiving HAART, 29 patients (52%) had undetectable viral load and 45 (80%) had a CD4$^+$ count >200/mm^3. At the last evaluation, 36 out of 38 patients (95%) were receiving HAART, 21 patients (55%) had undetectable viral load and 22 (81%) had a CD4$^+$ count >200/mm^3. None of the patients developed opportunistic infection during follow-up.

Effects of bosentan therapy on overall and event-free survival

At the cut off date (1st December 2007), the mean follow-up was 29±18 months (range 1-64 months). Forty-four patients were alive, including 36 receiving bosentan monotherapy and five receiving bosentan in combination with epoprostenol (n=1), iloprost (n=1), or sildenafil (n=3). The three patients for whom bosentan treatment was withdrawn following elevation of liver enzymes were still alive and were not receiving any specific PAH therapy. These three patients did not receive sildenafil because of a potential drug-drug interaction as they were receiving protease inhibitors at the time. In addition, these three patients did not receive intravenous

epoprostenol because they did not reach the criteria for intravenous therapy. Overall survival rates were 93±3%, 86±5% and 66±8% at 1, 2 and 3 years, respectively (Figure 1A). Most of the 15 deaths were not related to PAH; the causes of death were sepsis (n=6), complications of HIV infection (HIV-associated dementia) (n=1), gastrointestinal bleeding related to portal hypertension (n=2) or unknown cause (n=2). Four patients died as a result of right heart failure. One patient died during combination therapy with epoprostenol and bosentan; the remaining 14 patients died whilst receiving bosentan monotherapy. The rates of event-free survival were 82±5%, 73±6% and 54±8% at 1, 2, and 3 years, respectively (Figure 1B).

The results of univariate analysis of the relationship between overall survival and variables measured at baseline are shown in Table 4. History of right heart failure and baseline WHO FC IV were significantly related to overall survival. Co-infection with hepatitis B or C virus did not influence long-term survival. Similarly, the presence of mild cirrhosis did not affect outcomes.

Discussion

This study investigated a large number of consecutively-enrolled patients with PAH-HIV treated with first-line bosentan and confirms the short-term clinical and haemodynamic improvements observed in a previous 16-week pilot study.[17] In addition, the observed short-term improvements were sustained long-term in the majority of patients, resulting in favourable survival estimates. Moreover, long-term treatment with bosentan combined with HAART was well tolerated and was not associated with adverse effects or impaired control of HIV infection.

The mean treatment duration of 29 months allows an adequate evaluation of the potential long-term benefits of bosentan therapy in these patients. The survival rates of 93%, 86% and 66% at years 1, 2 and 3, respectively, are better than those observed in previous series of patients with PAH-HIV.[5,6] Petitpretz *et al.* described a 2-year survival of 46% in 20 PAH-HIV patients receiving supportive therapy alone.[6] In a group of 82 patients receiving supportive therapy either alone (n=62) or in combination with epoprostenol (n=20), Nunes *et al.* reported overall survival rates of 73%, 60% and 47% at 1, 2 and 3 years, respectively.[5] Using univariate analysis, we found that patients in WHO FC IV at baseline and patients with a history of heart failure had an increased risk of mortality. This highlights the importance of screening patients with HIV infection and unexplained dyspnoea in order to start PAH-specific therapy early in the course of the disease before the occurrence of right heart failure.[2] In contrast with the study by Nunes *et al*. where 75% of deaths were related to pulmonary hypertension, we found that most deaths in our population were related to infectious complication or bleeding in the context of portal hypertension.[5,6] Nunes *et al.* identified on multivariate analysis that the $CD4^+$ lymphocyte count was an independent predictor of better survival.[5] In our study, univariate analyses indicate that patients treated with HAART and patients with

undetectable HIV viral load tend to have a better prognosis. We did not identify haemodynamic parameters as predictors of survival, perhaps indicating that the beneficial effect of bosentan on haemodynamic parameters may have considerably changed the natural history of PAH-HIV.

Of considerable interest is the normalisation of haemodynamic parameters in 10 of the 59 patients who received bosentan monotherapy. This was considered not to result from less severe disease, as patients with or without normalised haemodynamics had similar functional and haemodynamic parameters and comparable HIV status at baseline. This finding is somewhat surprising because complete normalisation of haemodynamic parameters is exceptional with PAH-specific therapy (i.e. prostanoids and/or endothelin receptor antagonist and/or PDE5i) in patients with either IPAH or PAH associated with concomitant disease.[19] Some patients with PAH associated with some inflammatory diseases, such as systemic lupus erythematosus or mixed connective tissue disease, may achieve normalised haemodynamics with corticosteroids and/or immunosuppressive therapy. Recently, Montani *et al.* reported a case of sustained normalisation in haemodynamics in a patient with co-morbid HIV infection, human herpes virus 8 infection and Castleman's disease 5 years after a 12-month treatment combining cyclophosphamide, corticosteroids, HAART and epoprostenol.[24] In our series, bosentan was withdrawn in a single patient who had a sustained 2-year normalisation of haemodynamics whilst on bosentan. This patient remained stable 6 months after bosentan withdrawal.

While the pathogenesis and exact mechanism of development of PAH in patients with HIV have not been clearly defined, the benefits of bosentan suggest a key role of

endothelin in this process. HIV is likely to play a role in the pathogenesis of PAH through the release of mediators associated with retroviral infection, namely platelet-derived growth factor and endothelin-1.[25] HIV viral antigens are present in pulmonary endothelium and may directly stimulate abnormal apoptosis, growth, and proliferation. Thus, glycoprotein 120, a viral protein necessary for the binding and entry of HIV into macrophages, has been shown to target human lung endothelial cells, increase markers of apoptosis and stimulate the secretion of endothelin-1.[26] It has been shown that the membrane-trafficking regulator Nef increase apoptosis followed by proliferation of endothelial cells in vitro.[27] Finally, the transcriptional transactivator tat protein, has been shown to stimulate endothelial cell proliferation resulting in the release of growth factors.[27]

The management of PAH in HIV-infected patients is particularly challenging given the complexity and frequent changes of antiretroviral drug regimens. As a consequence, it is difficult to know if a theoretical drug-drug interaction between bosentan and antiretroviral drugs may have had clinically-relevant consequences in the population studied here, and this study was not designed to address this question. Despite this, the number of patients who were receiving HAART, who had undetectable viral loads and whose $CD4^+$ lymphocyte counts were $<200/mm^3$ is comparable to overall national data[28] and did not change with bosentan treatment.

Only three patients in this study withdrew from bosentan treatment due to elevations of liver enzymes. Such elevations are a class effect of endothelin receptor antagonists. In a recent large-scale post-marketing surveillance, the frequency of liver enzyme elevations was no higher in HIV-infected patients than in patients with PAH due to other etiologies.[29]

The major limitation of the present study is its retrospective nature. Moreover, long-term functional and haemodynamic data on the effect of bosentan in

this patient population may have introduced selection bias since the analysis may have concentrated only on survivors or on patients who did not experience clinical deterioration and thus remained on monotherapy. Nevertheless, bosentan appeared to be at least as effective for treatment of PAH in HIV-infected patients as previously observed in double-blind, placebo-controlled studies in patients with IPAH and associated PAH[30] as well as the longer-term open-label extensions of these studies.[31] Moreover, there are currently ethical reasons for not performing a controlled study of specific PAH therapies against placebo in patients with PAH-HIV given their poor prognosis with supportive therapies alone (e.g. anticoagulants, diuretics, oxygen).[5,6] Nevertheless, the availabilities of HAART has significantly improved the prognosis and quality of life of people living with HIV.

In summary, this study indicates that the endothelin receptor antagonist bosentan provides long-term clinical and haemodynamic improvements, as well as a possible favourable impact on survival in HIV-infected patients with PAH. Bosentan was observed to be safe in conjunction with HAART with no adverse effects on the control of HIV infection. The oral route of administration of bosentan is advantageous by comparison with that of parenteral prostanoids.

Acknowledgements

Editorial support was provided by Elements Communications Ltd on behalf of Actelion Pharmaceuticals France.

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Figure Legends

Figure 1A:

Estimated survival rates were 93%, 86% and 66% at 1, 2, and 3 years, respectively.

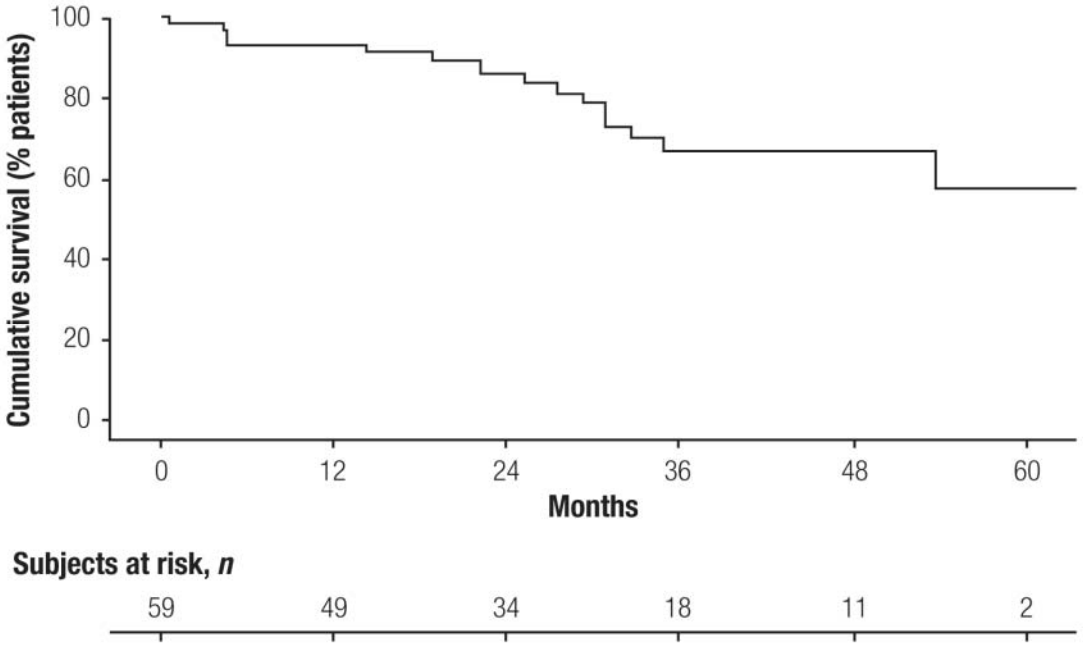
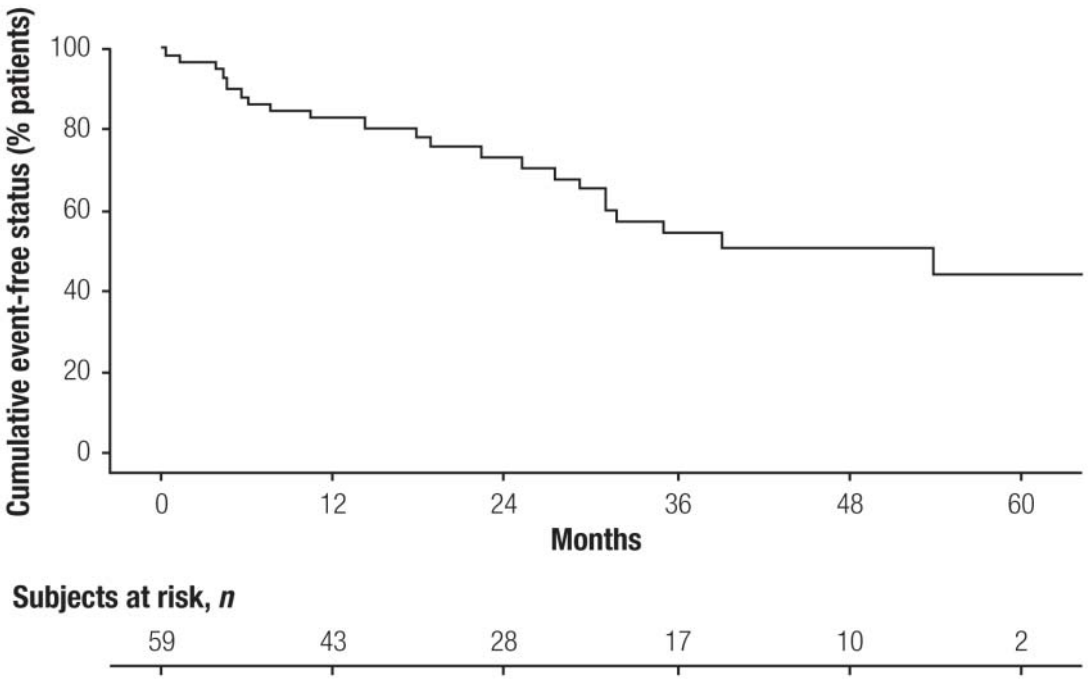


Figure 1B:

Estimates of event-free status were 82%, 73% and 54% at 1, 2, and 3 years, respectively.



Subjects at risk, *n*

59	43	28	17	10	2

Figure 1A: Kaplan-Meier estimates of survival in 59 patients with pulmonary arterial hypertension associated with HIV infection treated with first-line bosentan therapy

Figure 1B: Kaplan-Meier estimates of event-free status in 59 patients with pulmonary arterial hypertension associated with HIV infection treated with first-line bosentan therapy

Table 1: Clinical characteristics of the study population at baseline (n=59)

Demographic	
Gender: Men: Women, n (%)	37 (63%): 22 (37%)
Age, yr	40±15
Weight, kg	63±16
Ethnic group: Caucasian: Black, n (%)	50 (85%): 9 (15%)
WHO FC I : II : III : IV, n	0 : 4 : 49 : 6
6-minute walk distance, m	355±99
HIV status	
CDC stage, n (%)	
Asymptomatic - A	30%
Symptomatic non-AIDS - B	9%
Symptomatic AIDS - C	61%
$CD4^+$ lymphocyte count, cells/mm^3	437±574
$CD4^+$ lymphocyte count >200/mm^3, n (%)	47 (80%)
Undetectable viral load, n (%)	30 (51%)
HAART, n (%)	49 (83%)
Haemodynamics	
mRAP, mmHg	8±5
mPAP, mmHg	49±10
mPCWP, mmHg	6±2

Cardiac index, $L \cdot min^{-1} \cdot m^{-2}$	2.9±0.7
PVR, $dyn \cdot s \cdot cm^{-5}$	749±321
SvO_2, %	60±9
Acute responder to inhaled nitric oxide*, n	0

Values are expressed as mean±SD.

AIDS: Acquired immunodeficiency syndrome; CDC: Center for Diseases Control and Prevention; FC: functional class; HAART: Highly active antiretroviral therapy; mBP: mean blood pressure; mPAP: mean pulmonary artery pressure; mPCWP: mean pulmonary capillary wedge pressure; mRAP: mean right atrial pressure; WHO: World Health Organization; PVR: pulmonary vascular resistance; SvO_2: mixed venous oxygen saturation.

*An acute response to inhaled nitric oxide was defined as a fall in mPAP of >10 mmHg to reach a value <40 mmHg with a normal or elevated cardiac output.[7,18]

Table 2: Exercise capacity, haemodynamics and HIV status on bosentan monotherapy

	Baseline	4 months
	(n=56)	
WHO FC I : II : III : IV, n	0 : 4 : 45 : 7	9 : 36 : 11 : 0
6-minute walk distance, m	358±98	435±89†
mRAP, mmHg	8±5	5±3†
mPAP, mmHg	49±10	40±13†
Cardiac index, $L \cdot min^{-1} \cdot m^{-2}$	2.9±0.7	3.5±0.8†
PVR, $dyn \cdot s \cdot cm^{-5}$	737±328	476±302†
SvO_2, %	60±9	66±7†
Viral load, log_{10}/mm^{-3}	2.2±2.3	2.0±2.1
$CD4^+$ cell count, $cells/mm^3$	379±296	422±301

	Baseline	4 months	Last evaluation§
		(n=38)	
WHO FC I : II : III : IV, n	0 : 2 : 34 : 2	6 : 25 : 7 : 0	11 : 18 : 9 : 0
6-minute walk distance, m	357±80	424±82†	449±91
mRAP, mmHg	9±5	6±3†	6±4†
mPAP, mmHg	50±10	40±13†	37±15†‖
Cardiac index, $L \cdot min^{-1} \cdot m^{-2}$	2.8±0.6	3.5±0.7†	3.6±0.9†

PVR, dyn·s·cm^{-5}	769±355	470±285†	444±356$^{\dagger\,\|\|}$
Viral load, log_{10}/mm^{-3}	2.3±2.4	2.0±1.9	1.2±1.5$^{\dagger\,\|\|}$
CD4^{+} cell count, cells/mm^{3}	391±316	399±291	454±311$^{\dagger\,\|\|}$
SvO_2, %	59±9	66±7†	65±8†

Values are expressed as mean±SD.

FC: functional class; mPAP: mean pulmonary artery pressure; mRAP: mean right atrial pressure; WHO: World Health Organization; PVR: pulmonary vascular resistance; SvO_2, mixed venous oxygen saturation

§ last evaluation performed 29±15 months after bosentan initiation (range 12-67 months)

† P<0.005 *vs* baseline; $^{\|\|}$ P<0.05 *vs* 4-month.

Table 3: Individual data of the 10 patients who normalised haemodynamics on bosentan monotherapy.

	Baseline						Last evaluation (32±22 months)					
Patient N°	6MWD (m)	mPAP (mmHg)	CI ($L.min^{-1}.m^{-2}$)	PVR ($dyn.s.cm^{-5}$)	Viral load (log_{10}/mm^3)	$CD4^+$ count ($cells/mm^3$)	6MWD (m)	mPAP (mmHg)	CI ($L.min^{-1}.m^{-2}$)	PVR ($dyn.s.cm^{-5}$)	Viral load (log_{10}/mm^3)	$CD4^+$ count ($cells/mm^3$)
1	300	51	3.6	667	0	271	577	13	2.9	114	0	691
2	390	46	3.3	578	5.8	127	460	14	3.5	146	2.5	597
3	420	46	2.1	1075	5.0	200	505	15	4.8	108	3.0	202
4	362	62	3.4	830	0	282	535	17	4.5	77	0	420
5	280	51	3.5	529	0	431	575	17	3.7	136	0	398
6	403	47	3.2	577	0	205	538	18	4.4	123	0	616
7	547	39	4.5	278	0	280	620	21	5.5	112	0	471
8	422	48	4.1	385	0	430	450	24	4.1	200	2.7	501
9	450	47	3.0	923	0	450	540	24	4.0	272	0	650
10	180	74	2.3	1288	0	363	520	24	3.4	225	0	842
Mean	375	51	3.3	713	1.1	304	532*	19*	4.1*	151*	0.8	539*
SD	102	10	0.7	314	2.3	111	52	4	0.8	61	1.3	180

6MWD: 6-minute walk distance; CI: cardiac index; mPAP: mean pulmonary artery pressure; PVR: pulmonary vascular resistance; SD: standard deviation

*: $p<0.05$ vs. Baseline

Table 4: Result of Cox regression analysis relating prognostic factors for mortality to individual selected variables at baseline

Variables	Hazard ratio (95% confidence limits)*	P-value
Gender, female : male	0.51 (0.14 - 1.84)	0.32
History of right heart failure, yes : no	3.11 (1.12 - 8.62)	0.03
Co-infection with HBV or HCV, yes : no	0.85 (0.30 - 2.39)	0.76
Portal hypertension, yes : no	0.30 (0.04 - 2.32)	0.25
WHO FC, IV : III	4.58 (1.41 - 14.85)	0.02
6-minute walk distance <368m, yes : no	1.00 (0.34 - 2.91)	0.90
Undetectable viral load, yes : no	0.25 (0.06 - 1.13)	0.07
$CD4^+$ lymphocyte count >282/mm^3, yes : no	0.49 (0.17 - 1.37)	0.17
HAART, yes : no	0.32 (0.10 - 1.03)	0.06
mRAP >6 mmHg, yes : no	1.69 (0.53 - 5.26)	0.37
mPAP >47 mmHg, yes : no	0.79 (0.28 - 2.17)	0.64
Cardiac index <2.93 $L \cdot min^{-1} \cdot m^2$, yes : no	3.24 (0.91 - 11.52)	0.07
PVR >702 $dyn \cdot s \cdot cm^{-5}$, yes : no	1.85 (0.62 - 5.26)	0.27

FC: functional class; HAART: highly active antiretroviral therapy; HBV: hepatitis B virus; HCV: hepatitis C virus; mPAP: mean pulmonary artery pressure; mRAP: mean right atrial pressure; WHO: World Health Organization; PVR: pulmonary vascular resistance.

* Values of hazard ratio >1 indicate an increased risk of death.